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Developmental Biology 306 (2007) 396–410

DEVELOPMENTAL BIOLOGY

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Abstracts

Early embryo patterning

Program/Abstract # 287

Plasmin formation during differentiation of the implanting mouse blastocyst

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As the blastocyst implants into the uterine wall, proteolytic enzymes are required for endometrial remodeling. A proteolytic cascade initiated by transformation of plasminogen to plasmin degrades extracellular matrix (ECM) and activates matrix metalloproteinases, which are directly responsible for the catabolism of ECM. Our objective was to evaluate whether plasminogen is processed to plasmin by mouse peri-implantation embryos. Blastocysts explanted on gestation day (GD) 4 were cultured in microdrops of Ham F10 medium supplemented with 0.1% BSA. After incubation for 2 days with 10 µg/mL plasminogen, plasmin was analyzed by zymography in SDS gels co-polymerized with casein. Several caseinolytic bands were detected by zymography, including two of 84 and 87 kDa, which were also adsorbed to the culture surface. Other bands of 65, 50, 48, 40, 39 and 36 kDa were detected in the conditioned medium. The low molecular weight bands could be generated from commercially obtained purified plasmin that initially had zymographic bands of 84 and 87 kDa by incubation at 37 °C for 48 h, suggesting that they were autolytic products of plasmin. Also, after addition of plasminogen to the medium conditioned by cultured embryos from GD 5 to 9, plasmin activity was detected by an amidolytic assay. In conclusion, mouse blastocysts developing in vitro are able to process plasminogen into several plasmin isoforms to support tissue remodeling during embryo implantation and early placentation. Supported by PAPCA 2007, FES-Iztacala, UNAM.

doi:10.1016/j.ydbio.2007.03.588

Program/Abstract # 288

The role of siamois and twin in organizer gene induction

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In early *Xenopus laevis* development, the Spemann organizer regulates the patterning of the mesoderm at the marginal zone and is essential for the establishment of a complex and organized body plan. Overlap of the domains of active Wnt and Nodal signaling in the dorsal vegetal region of the embryo is essential for organizer formation. The transcription factors *siamois* (Sia) and *twin* (Twn) are expressed in the dorsal vegetal blastomeres in response to stabilized beta-catenin and are thought to be key regulators of organizer gene expression. Sia and Twn are among the earliest organizer genes expressed following midblastula transition and have been shown to be essential for organizer formation and induction of goosecoid (Gsc) in the organizer. In Sia, amino acids 40–75 are critical for its transcriptional activation; in Twn, the activation domain maps to a similar position. The activation domain is necessary for Sia and Twn mediated induction of organizer gene expression and secondary axis formation. Chromatin immunoprecipitation has revealed that Sia and Twn bind the Gsc promoter *in vivo*; ongoing experiments are focused on identifying co-factors which cooperate with Sia and Twn in Gsc induction. Other work focuses on how Sia and Twn are cooperating with members of the Nodal signaling pathway to induce expression of organizer-specific genes. Defining the mechanism by which Sia and Twn function to promote the formation of the organizer is critical to understanding how the overlap of two distinct signaling pathways cooperate to establish the organizer.

doi:10.1016/j.ydbio.2007.03.589

Program/Abstract # 289

Early development of the foam-nesting frogs *Engystomops randi* and *E. coloradorum*

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doi:10.1016/j.ydbio.2007.03.313

We have studied the embryos of two foam nesting frogs, *Engystomops randi* and *E. coloradorum* (Leiuperidae) from fertilization to tadpole hatching and prepared a normal table of development, which was divided into 23 stages. The foam nests, built on standing water, each contain about 200 white eggs (1.1 and 1.3 mm in diameter, respectively) that resemble *X. laevis* albino eggs and develop at almost the same rate. Embryo morphology was evaluated in whole mount preparations and vibratome sections. Somites were analyzed morphologically and by immunostaining with an anti-myosin antibody. Although both species approach the egg size and developmental rates of *X. laevis*, their development differs in significant ways, an aspect that may relate to the different breeding strategies of these frogs. The morphology of cleavage and gastrulation resembles the *X. laevis* developmental pattern. Similarly, elongation of the archenteron and notochord begins before blastopore closure. At later stages, however, embryos of *Engystomops* differ greatly from *X. laevis* not only in the general shape of the embryo at the tailbud stage, but also in the pattern of somite differentiation. The first somites were detected in the mid-neurula instead of in the late gastrula. The pattern of myotome differentiation includes the intercalation of numerous cells, similar to that which occurs in *Gastrotheca riobambae* and *Bombina variegata*. This pattern greatly differs from myotome differentiation in *X. laevis*. This is the first description of early development in frogs of the genus *Engystomops*.

doi:10.1016/j.ydbio.2007.03.590

Program/Abstract # 290
Gastrulation in four species of dendrobatid frogs
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We have studied gastrulation of four closely related species of dendrobatid frogs, *Epipedobates anthonyi*, *Epipedobates tricolor*, *Epipedobates ingeri* and *Dendronbates auratus*. Developmental patterns have been compared with *Xenopus laevis* and a marsupial frog, *Gastrotheca riobambae*. Gastrulation morphology was analyzed in whole mount and in cross-sections stained for cell nuclei. We observed great variability in egg size amongst the dendrobatids (egg diameters, 1.6 to 3.5 mm). Eggs of some dendrobatids are larger than those of *G. riobambae* (3 mm in diameter). *G. riobambae* has one of the most divergent patterns of frog gastrulation, with the formation of an embryonic disk. Gastrulae of most dendrobatid frogs share with *G. riobambae* a delayed elongation of the archenteron and the formation of a large circumblastoporal collar. We therefore asked whether the formation of an embryonic disk is associated with egg size. The results show that despite the large egg size, there are similar features of gastrulation among dendrobatids, for example gastrulation time, time of archenteron expansion, thickness of the roof of both the blastocoele and archenteron and the degree of cell accumulation in the blastopore lip. Nevertheless, an embryonic disk was not formed. Egg size variation and similarity of gastrulation processes suggest that the gastrulation pattern is highly conserved among dendrobatids, and that egg size is not related to the formation of an embryonic disk. Gastrulation in dendrobatids is a useful subject for further study, as it greatly differs from *X. laevis* gastrulation.

doi:10.1016/j.ydbio.2007.03.591

Program/Abstract # 291
SAGE analysis of dorsal and ventral transcriptome of *Xenopus tropicalis gastrula*
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Xenopus has been a favourable model for studying gene function during early embryonic development. Substantial progress has been made in the identification of genes and molecular mechanisms involved in dorsoventral patterning of *Xenopus* embryos. However, all these pre-genomic approaches were generally biased to the detection of a limited group of transcripts. In addition, global approaches in several species have demonstrated that transcriptomes are more complex than expected. At present, genomic approaches can be performed in *Xenopus tropicalis* because its diploid genome sequence is available. In this work, we used the global approach SAGE to identify novel genes involved in dorsoventral patterning of *Xenopus* embryos. SAGE permits a qualitative and quantitative analysis of the transcriptomes and the detection of novel transcripts. SAGE libraries were prepared from dorsal and ventral explants of *Xenopus tropicalis* gastrula and 30,000 tags/library have been obtained. Here, we present the comparative analysis of these libraries. We performed tag-mapping by using both genome sequence and known transcripts because ~35% of the experimental tags have no match in known transcripts databases. We modified a novel bioinformatics method, named *Hierarchical Gene Assignment*, for proper tag-mapping in this genome. We have begun to experimentally verify tag assignments by RT-PCR and novel transcripts identified will be used for functional studies. This is the first SAGE experiment in *Xenopus tropicalis* and we expect to find novel genes involved in dorsoventral patterning.

doi:10.1016/j.ydbio.2007.03.592

Program/Abstract # 292
Syndecan-4 in non-canonical Wnt signaling and gastrulation movements in *Xenopus* embryos
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